Controlled oxidation of thiols to disulfides by diazenecarboxamides

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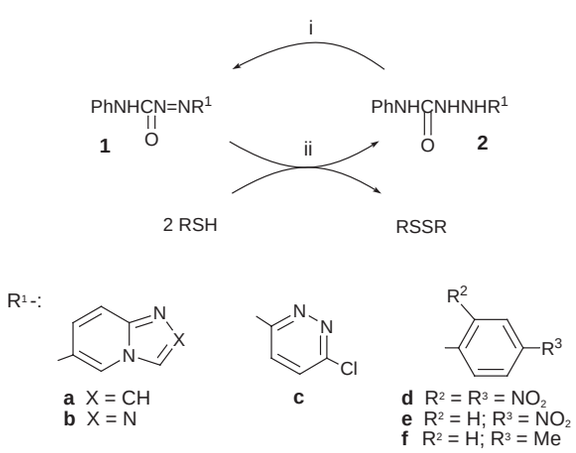
COMMUNICATION

New reagents were developed and were found to be convenient and selective oxidants of various thiols (including glutathione, cysteamine and dithiothreitol) to disulfides under mild reaction conditions.

Disulfides are of great importance in chemistry as well as in biochemistry. Sulfur–sulfur bonds between cysteine residues stabilise three-dimensional structures of many peptides, hormones and toxins.[1] Several synthetic disulfides were tested as tyrosine kinase inhibitors.[2] Up to now, there is, to the best of our knowledge, only a limited number of reagents which could serve as candidates for the oxidation of a thiol to a disulfide *in vivo*.[3] Using diazenecarboxylic acid esters and diazenedicarboxamides, Kosower showed some interesting results on several biological systems.[4] Unfortunately, most of the diazenes were found to be unstable.[4b,5]

Having those results in mind as well as the fact that amides are more stable towards hydrolysis than the corresponding esters, diazenecarboxamides **1** were thought to be suitable target compounds for the thiol oxidation. Unsymmetrical diazenecarboxamides were selected as they possess substituents, directly linked to the diazene moiety, which could exhibit a donating or withdrawing effect to the N=N bond. Our attention has recently been devoted to various transformations of hydrazides or their derivatives,[6] and diazenes were presumed as intermediates in the oxidation of a hydrazino moiety by thallium(III) nitrate (TTN)[7] or by ceric ammonium nitrate (CAN).[8] Now, we would like to report that the oxidation of 1,4-disubstituted semicarbazides **2** with TTN[9] or CAN indeed resulted in the formation of diazenecarboxamides of type **1** (Scheme 1), which exhibited some interesting properties.



Scheme 1 *Reagents and conditions*: i, CAN or TTN, MeOH, room temp., 1–10 min, 83–98%; ii, MeOH, argon, room temp., 80–100%.

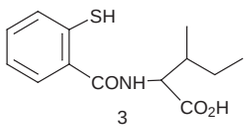
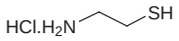
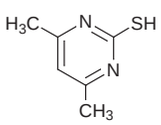
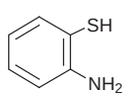
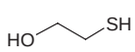
Diazenecarboxamides **1** were found to be stable in the solid state as well as in solutions of many organic solvents or water even after extended periods of time. Although they were fully characterised by NMR, IR and elemental analysis, their mass spectra showed under EI conditions more abundant $(M + 2)^+$ signals than the expected molecular peaks (M^+). Such behaviour indicated diazenecarboxamides **1** as acceptors of two hydrogen atoms and therefore as possible oxidants. A number of experiments, which were performed with different **1**, clearly demonstrated that there was no reaction on treatment with a variety of amines, aldehydes, dihydronaphthalenes or carboxylic acids. On the other hand, diazenes **1** did react with thiols to give disulfides. For example, diazenes **1a**–**1d** oxidised thiophenol (MeOH, argon, room temp.) within 5 minutes, giving diphenyl disulfide and the corresponding semicarbazide **2** as the only products. Furthermore, diazene **1e** reacted completely in 1 h, while more than one third of **1f** remained unchanged on treatment with thiophenol after 24 h.

The above results suggested that the group R^1 of the diazenecarboxamide **1** influenced its electrophilic character. Therefore, a diazene bearing an electron-withdrawing group should be a more powerful oxidant than that possessing an electron-donating substituent. It was in fact supported by the reactivity of **1d**–**1f** towards thiophenol. The nature of the substituent R^1 also seemed to influence the electrochemical properties of diazenes **1**. For that reason we performed cyclic voltammetry (CV) on diazenecarboxamides **1**; they were reduced in a single step, which corresponded to the reduction of the diazene group. The process was in all cases an irreversible one, therefore standard redox potentials (E^o) could not be determined.[10] On the other hand, cathodic peak potentials (E_{pc}) were easily obtained from cyclic voltammograms (cathodic peak potentials *vs.* Ag/AgCl, *i.e.* +197 mV *vs.* SHE; E_{pc}: **1a**, −256 mV; **1b**, −164 mV; **1c**, −212 mV; **1d**, −96 mV; **1e**, −264 mV; **1f**, −518 mV).[11] A comparison of E_{pc} values of **1a**–**1f** led to the following conclusion: a diazene, possessing a more positive (or less negative) cathodic peak potential bears a more electron-withdrawing group R^1, directly attached at the N=N bond.

The most effective diazenes were then applied for the oxidation of several selected thiols: glutathione (GSH), a tripeptide, which is present in all living cells and plays an important role in biological redox systems;[1c,12] thiosalicylic acid derivative **3**, whose oxidised form was recently used as one of the most promising anti-HIV agents;[13] cysteamine, dithiothreitol (DTT), and others (Table 1). Glutathione was also treated with diazene **1a** (or **1b**) under quasi-physiological conditions (0.15 M aqueous solution of KCl, pH 7.4, presence of air, concentration of glutathione: 10 mmol dm^{-3}, an equimolar amount of the selected diazene, 30 °C): oxidised glutathione (GSSG) and semicarbazide **2a** (or **2b**) appeared quantitatively within 90 min (15 min in the case of **1b**) as evident from NMR. It should be mentioned that glutathione remained unchanged after 90 min under the same conditions in the absence of a diazene.

In conclusion, our preliminary studies represent diazenecarboxamides **1** as a new type of convenient reagents for a controlled oxidation of thiols to disulfides. Reactions proceed smoothly, under extremely mild conditions and are highly chemoselective. Semicarbazides **2**, which are formed along with disulfides, can be reoxidised to **1**. Diazenecarboxamides **1** are stable in the presence of nucleophiles, including water, in contrast to their structural analogues, diazenecarboxylic acid esters. Cathodic peak potentials indicate the oxidative efficiency of **1** and can easily be tuned by the choice of the appropriate substituents. Diazenes of type **1** may find a plethora of applications, not only for synthetic purposes, but also in connection with enzyme activity,[1c,12] and the activity of a transcription factor.[14]

Table 1 Oxidation of selected thiols with diazenes[a]

Thiol	Diazene	Time/min	Disulfide[b] (%)
GSH[c]	**1a**	110	99
 3	**1b**	2	81 (100[d])
	**1a**	1	90
DTT	**1d**	150	100[d]
	**1c**	10	90
	**1c**	5	99
	**1a**	1	80

[a] MeOH, argon, room temp. [b] Isolated yields are given. [c] H_2O–MeOH (1:1, 10 cm^3) was used as a solvent. [d] Quantitatively according to ^1H NMR analysis.

Experimental

Oxidation of semicarbazides 2 with ceric ammonium nitrate (CAN)

To a stirred suspension of a selected **2** (2 mmol) in MeOH (5 cm^3), a solution of CAN (2.30 g, 4.2 mmol) in MeOH (10 cm^3) was added dropwise at room temperature. After additional stirring for 5–10 minutes at room temperature, water (30 cm^3) was added and the precipitated **1** was collected by filtration and crystallized from the appropriate solvent.

1a. Mp 183–183.5 °C (MeOH); δ_H (300 MHz, DMSO-d_6) 7.22 (m, 1H, *p*-Ph), 7.45 (m, 2H, *m*-Ph), 7.65 (d, *J* 9.7 Hz, 1H, H-7), 7.79 (m, 2H, *o*-Ph), 8.02 (d, *J* 1.3 Hz, 1H, H-2), 8.41 (dd, J_1 9.7 Hz, J_2 0.7 Hz, 1H, H-8), 8.58 (dd, J_1 1.3 Hz, J_2 0.7 Hz, 1H, H-3), 11.23 (br s, 1H, NH); δ_C (75 MHz, DMSO-d_6) 107.84, 118.29, 119.55, 124.74, 127.43, 129.07, 136.17, 137.56, 138.89, 157.78, 158.72; *m*/*z* (EI) 268 (M^+ + 2, 3.5%) (Found: C, 58.32; H, 3.53; N, 31.86. Calcd. for $C_{13}H_{10}N_6O$: C, 58.64; H, 3.79; N, 31.56%).

1b. Mp 190–193 °C (MeOH); δ_H (300 MHz, DMSO-d_6) 7.22 (m, 1H, *p*-Ph), 7.45 (m, 2H, *m*-Ph), 7.71 (d, *J* 9.9 Hz, 1H, H-7), 7.77 (m, 2H, *o*-Ph), 8.63 (dd, J_1 9.9 Hz, J_2 0.7 Hz, 1H, H-8), 9.96 (d, *J* 0.7 Hz, 1H, H-3), 11.32 (br s, 1H, NH); δ_C (75 MHz, DMSO-d_6) 112.35, 119.57, 124.90, 127.02, 129.11, 137.40, 140.32, 143.51, 158.25, 159.07; *m*/*z* (EI) 269 (M^+ + 2, 8%) (Found: C, 53.63; H, 3.22; N, 36.48. Calcd. for $C_{12}H_9N_7O$: C, 53.93; H, 3.39; N, 36.69%).

1c. Mp 193–195 °C (MeOH); δ_H (300 MHz, DMSO-d_6) 7.22 (m, 1H, *p*-Ph), 7.45 (m, 2H, *m*-Ph), 7.77 (m, 2H, *o*-Ph), 8.09 (d, *J* 9.1 Hz, 1H), 8.27 (d, *J* 9.1 Hz, 1H), 11.27 (br s, 1H, NH); δ_C (75 MHz, DMSO-d_6) 119.59, 120.75, 124.82, 129.09, 131.91, 137.43, 158.51, 158.56, 164.50; *m*/*z* (EI) 263 (M^+ + 2, 1.2%) (Found: C, 50.76; H, 2.81; N, 26.76. Calcd. for $C_{11}H_8ClN_5O$: C, 50.49; H, 3.08; N, 26.76%).

1d. Mp 165–167 °C (MeOH–DMF); δ_H (300 MHz, DMSO-d_6) 7.22 (m, 1H, *p*-Ph), 7.45 (m, 2H, *m*-Ph), 7.77 (m, 2H, *o*-Ph), 7.82 (d, *J* 8.8 Hz, 1H), 8.74 (dd, J_1 8.8 Hz, J_2 2.4 Hz, 1H), 9.03 (d, *J* 2.4 Hz, 1H), 11.40 (br s, 1H, NH); δ_C (75 MHz, DMSO-d_6) 119.52, 120.15, 120.54, 124.95, 129.14, 129.17, 137.35, 146.07, 147.36, 148.79, 157.45; *m*/*z* (EI) 317 (M^+ + 2, 2%) (Found: C, 49.61; H, 2.93; N, 22.27. Calcd. for $C_{13}H_9N_5O_5$: C, 49.53; H, 2.88; N, 22.22%).

1e. Mp 160–162 °C (MeOH); δ_H (300 MHz, DMSO-d_6) 7.20 (m, 1H, *p*-Ph), 7.43 (m, 2H, *m*-Ph), 7.75 (m, 2H, *o*-Ph), 8.14 (m, 2H), 8.50 (m, 2H), 11.11 (br s, 1H, NH); δ_C (75 MHz, DMSO-d_6) 119.47, 124.00, 124.62, 125.15, 129.04, 137.61, 149.68, 154.13, 159.07; *m*/*z* (EI) 272 (M^+ + 2, 2.5%) (Found: C, 57.91; H, 3.46; N, 20.93. Calcd. for $C_{13}H_{10}N_4O_3$: C, 57.78; H, 3.73; N, 20.73%).

1f. Mp 125–127.5 °C (MeOH–H_2O); δ_H (300 MHz, DMSO-d_6) 2.44 (s, 3H, Me), 7.17 (m, 1H), 7.37–7.50 (m, 4H), 7.77 (m, 2H), 7.86 (m, 2H), 10.87 (br s, 1H, NH); δ_C (75 MHz, DMSO-d_6) 21.10, 119.39, 123.18, 124.23, 128.92, 130.12, 138.02, 144.22, 149.21, 159.95; *m*/*z* (EI) 241 (M^+ + 2, 2.5%) (Found: C, 70.46; H, 5.44; N, 17.57. Calcd. for $C_{14}H_{13}N_3O$: C, 70.28; H, 5.48; N, 17.56%).

Typical procedure for the oxidation of thiols with diazenecarboxamides 1

A mixture of diazenecarboxamide **1** (0.5 mmol) and thiophenol (110 mg, 1.0 mmol) in MeOH (15 cm^3) was stirred under argon at room temperature until only diphenyl disulfide and semicarbazide **2** were detected in the reaction mixture (TLC evidence). Solvent was evaporated under reduced pressure, the products were separated by a short column (1.5–3 cm) of silica gel (220–440 mesh), using cyclohexane and then ethyl acetate, affording diphenyl disulfide and semicarbazide **2**. Oxidations of other thiols with **1** were carried out under the same reaction conditions as described for the thiophenol, only isolations of the products were slightly modified.

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